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Gene Therapy: Front runner in future medicine

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Abstract
Widely perceived earlier as a future medicine, Gene Therapy has progressed at a pace never before and has become today's reality. With earlier conception of limited applicability in genetic diseases only, this technique has now being explored for metabolic and physiological disorders too. More than a dozen diseases are being targeted with this approach, few products are in use and encouraging rate of success has been achieved.

This science is at the intersection of genetics, molecular biology and genetic engineering, gaining powerful addendums with increasing knowledge and innovative techniques, updated each passing day. With a myriad of options available for targeting cells, delivery of functional gene, integration and novel strategies such as Genetically Modified probiotics, it has greatly enhanced prospects.

A systematic progress in knowledge and careful integration of inter-disciplinary sciences have given the main boost. With the completion and annotation of Human Genome Project, it has become even more powerful and effective for a range of diseases, leading way towards realistic personalized medicine. Although, theoretically well planned and strict safety measure are taken while design, use of vectors and the foreign gene, but abrupt setbacks are also a reality. This review addresses different aspects of Gene Therapy and also on novel methods of transfer such as GM Probiotics, as the delivery agent.

Keywords: Gene Therapy, Retrovirus, Personalized medicine, Probiotics.

Introduction

Genes are the coding part of DNA which have the pivotal role of carrying forward the hereditary information and at the same time dictate all the physiological and metabolic processes, in interplay with the environment. Genes, code for all types of proteins and direct the lipid and carbohydrate metabolic pathways via their protein products – enzymes. Thus, they are rightfully the hereditary and functional units of heredity and life.

Being such a vital component of the cell, any changes (mutation) in its sequence and structure have grave consequences on body functioning, mainly manifested as genetic disorders. Recent advances in genetics, molecular biology and imaging techniques have brought a surge in detection of such disorders, even indicating more predisposition to cancer in many cases (Roth & Cristiano, 1997) [11]. Traditional therapies to mitigate such genetic diseases, such as drugs or other supplements which modulate cellular metabolism have proven either superficially effective or transiently active.

Recently, a new approach has been followed - Gene Therapy, which deals with faulty genes by augmentation by healthy copies of the same gene, introduced using specially designed targeted vectors. Originally invented to treat SCID (Severe Combined Immunodeficiency Syndrome) and cystic fibrosis disease, this concept has now been expanded to a dozen other genetic diseases, including the chronic ones (Calvo *et al.*, 2000; Kohn *et al.*, 2003) [16, 5].

The basis of Gene therapy is that disease condition can be reverted back or brought close to normal, if targeted genetic elements are introduced to augment the affected genes in diseased cells. Due to increased advancement in transfection strategies and recombinant DNA technology, many cell-specific and tissue specific vectors have been designed which have high transfection abilities and tunable integration into the host cell genome. This offers a range of choices to clinicians and scientists to access the situation and choose the right vector and DNA cassette to be transferred in the host (Bessis *et al.*, 2004) [20].

Although, Joshua Lederberg (1963) had introduced gene therapy, but Anderson (1990) popularized it. He got FDA approval to start ADA gene therapy inside WBCs (white blood cells) of a 4 year old patient, suffering from SCID. He observed good improvement in immune status of the patient. Later Rosenberg (1990), transfected neomycin resistance gene into metastatic lymphocytes (from 5 melanoma patients) using a retrovirus vector (Fischer *et al.*, 2013) [2]. The engineered recombinant lymphocytes were proliferated upto confluence *in vitro*

and transplanted back into the patients, confirming safety standards related to use of retrovirus for gene transfection. These initial successes prompted more than 1900 number of clinical trials using various gene transfection techniques for gene therapy. But, not all have met with success and there have been drastic setbacks in terms of only transient relief to patients and sometimes even death. Hence, the techniques used in gene therapy are yet to become hospital reality (Thomas *et al.*, 2003) [3].

China, USA & Europe form the epicenter for these studies and few more have flourished in Australia too. This strategy is most effective in conditions involving defective single gene which is recessive, such as in cystic fibrosis, muscular dystrophy, sickle cell anemia, hemophilia, viral diseases like AIDS and certain types of cancer. Few more target diseases which involve genetic susceptibility, such as diabetes mellitus, Alzheimer's disease, coronary heart disease and arthritis (Roth & Cristiano, 1997; Pearson *et al.*, 2004) [11, 26].

Gene Therapy - Design

Gene therapy is a complex and technically demanding area, thus the development of innovative techniques to target specific cell/tissues is time-taking and cumbersome. When designing a strategy for gene therapy, the metabolic and genetic requirement of the target cells must be considered and augmentation of defective gene should be targeted for its compensation of normal gene function. Simultaneously, efficient vectors for high and stable transfection should also be engineered for successful transfection into the target cell/tissue. Mostly, an active copy of a defective gene is transferred into host genome to augment its function. The technical difficulties in gene therapy are many, one of the foremost is the choice of vector for gene transfection (Kohn *et al.*, 2003; Bessis *et al.*, 2004; Thomas *et al.*, 2003) [5, 20, 3].

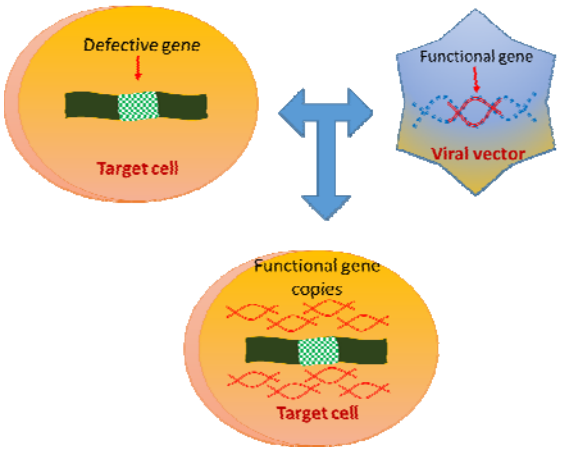

Fig 1: Basic work flow of Gene Therapy.

An ideal vector should have following characteristics:

1) **Copy number:** Maintenance/propagation in high copy number is prime prerequisite for a good vector.
2) **Specificity:** The vector should be cell/tissue specific and should be able to target regardless of cell's division status.
3) **Potency:** The vector should be non-immunogenic, able to transfer 1 or 2 genes and purifiable in high amounts.
4) **Patient/Environment safety:** the vector should not pose any threat to the patient and/or the environment.
5) **Stable transfection:** It should be capable of expressing the defective gene once activated and should be either stably integration or remain as episome.

A potent risk of insertional mutagenesis is always present in case of integrating vectors and thus a safer alternative is to use site-specific mutagenesis. Many regulatory elements are provided in the engineered gene to effectively control the expression timing and level of the gene (Xiao *et al.*, 1999) [28].

Classification

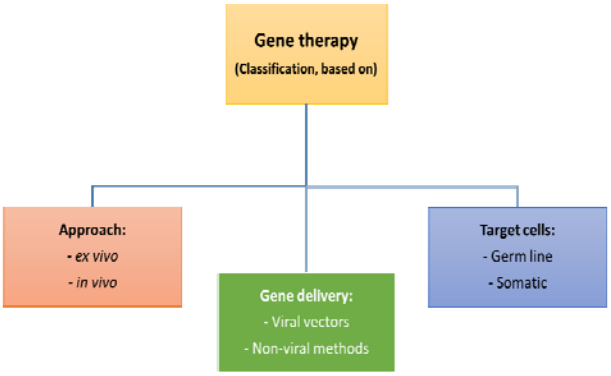

Fig 2: Classification of Gene Therapy based on different criterions.

Gene therapy has been practiced by using various approaches and can be bifurcated into:

1) *Ex vivo*

This approach follows harvesting target cells from healthy host and engineering it using transmission vectors. Then these vectors are transfected into harvested target cells and these recombinant cells are seeded back into host site (Porter *et al.*, 2011) [6]. This approach has benefit of being more organ-specific and have been found to be suitable for audio-visual pathway diseases (Bainbridge *et al.*, 2008) [12]. This approach has gained popularity in pre-clinical trials to treat blindness. This has also found applications in diseases such as retinal degeneration, corneal disease, stroke and multiple sclerosis, which might be due to lack of immune response in case of human optical system and modern neuro-protectants (Genovese *et al.*, 2014; Grossman *et al.*, 1994; Kalos *et al.*, 2014) [21, 17].

2) *In vivo*

This approach specially focuses on internal disorders as the previous approach cannot reach such depths. In this approach, in place of patient's cells, only the functional copies of the gene are injected directly into nearby tissue or bloodstream to augment the target site. Earlier clinical trials focused on genetic disorders, but this approach has been adapted lately for other conditions too such as allograft rejections, vascular interventions and atherosclerosis (Tebas *et al.*, 2014; Maruyama *et al.*, 2000) [22, 27].

The mainstay of Gene therapy lies with gene delivery and this can be broadly categorized as:

1) Viral vectors
2) Non-viral methods

The process of transfer of genes into host through viruses is called as "Transduction" while the methods not involving viruses are called as "Transfection".

1) Viral vectors

The viral vectors have high efficiency and have been used extensively (~70% of clinical trials). The vectors in use can be both non-integrating or integrating and thus pose host safety concerns too (Testa *et al.*, 2013; Nathwani *et al.*, 2011) [7, 1].

2) Non-viral methods
Naked DNA constructs which are transferred through non-viral modes such as microinjection, nanoparticles, liposomes, etc come under this category. Although less efficient, they are considerably safer than viral vectors and can be easily produced in high amounts.
Given the large variety of target host cells, Gene therapy can also be divided into (Sheridan, 2011) [4]:

1) Germ line
In this approach, functional genes are placed directly into germ cells to augment defective ones, so that it gets stably and heritably integrated in the genome too. Although very promising, but due to grave ethical concerns and technical prohibitions, use of such strategy has been prohibited in humans.

2) Somatic
In this approach, the somatic cells act as donor of the functional copies, and has been the most preferred and permissible mode in most countries. It has the drawback of the therapy not being a heritable.

Use of probiotics in Gene therapy
Probiotics are microbes that when taken in appropriate amounts (10^{7-9} CFU/ml) confers health benefit on the host (FAO, United Nations, 2001). Due to their GRAS (Generally Regarded as Safe) status and health benefits such as, maintenance of intestinal health, bowel movement, beneficial host immunomodulation and many more, they have been used extensively in the food and dairy sector (Martin *et al*., 2013) [25]. Thus, rather than using risk-prone viral vectors for gene therapy, focus has now shifted towards use of these beneficial microbes to deliver the functional copies to the affected site. Due to advances in gene manipulation technologies and better techniques available to transform these microbes (mostly Gram positive), they have found some application recently (Steidler, 2003; Geier *et al*., 2007; Sartor, 2004) [13, 19, 23]. In one such strategy, Thymine-minus mutant probiotic bacteria was used to deliver the human gene to the patient, which is technologically safe in being self-destructive after a certain time (when the thymine get depleted in the cell) and delivering the functional gene copies in its lifetime. In another strategy, recombinant *Escherichia coli Nissle 1917* containing a gene for Interleukin-10 was transfected to mitigate an experimentally induced Colitis in Mice, and considerable success was achieved (Gardlik *et al*., 2012) [24]. A European group used *Lactococcus lactis* as carrier to secrete ovalbumin (OVA) and demonstrated its ability to induce OVA specific immune tolerance in its T-cell receptor (TCR) containing mice (Huibregtse *et al*., 2007) [9]. Another group administered *Lactococcus lactis* having IL-10 gene in the gut and reported a 50% reduction in experimentally induced colitis in mice (Steidler *et al*., 2002). Recently, an innovative study reported that intravenous injection of *Bifidobacterium breve* – a probiotic shows pan-body distribution of the bacteria and specific accumulation in the cancerous tissue (Cronin *et al*., 2012) [15].

Risks Associated With Gene Therapy
As is said well, every therapy has side-effects and gene therapy too is no exception. This approach has actually seen many ups and downs than any other treatment procedure, due to lesser knowledge and drastic manifestations of this therapy. These concerns stem from use of viral vectors and even the naked DNA itself.
Of many risks associated with this therapy, here are a few:

1) Duration of Effect
For the augmented functional gene to show its effects, the targeted defective cell/tissue must stay alive. Also, due to a myriad of targets, techniques and transmission methods used, this technique suffers from being transient and slow.

2) Immunogenicity
Almost all viral vectors/naked DNA constructs pose a risk of heightened immune reaction, thus diminishing therapy efficiency and dosages.

3) Virulence hazard
Although, highly efficient, they pose as a hazard for cell toxicity, gene targeting and viruses might regain pathogenicity once established in the body.

4) Multi-locus diseases
Health disorders are not always due to single gene mutations, and many other diseases might prevail, making gene therapy of targeted gene complicated as a choice of treatment.

5) Insertional mutagenesis
Viruses might infect healthy cells during gene therapy and thus the transgene can gets integrated at untargeted locus disrupting that normal gene function, leading to a new disease.

6) Ethical considerations
Although, a feasible and powerful technology, gene therapy involves many unknown risks. Thus, strict adherence to standard protocol and continuous monitoring by the regulatory organizations is required throughout the experiment.

7) Epigenetics
It is the host-specific, environment controlled modulation of specific genes which dictate their functional status. Thus any prospective gene therapy would be complicated and mostly ineffective without taking epigenetic status in consideration.

Conclusion
Gene therapy has widely been accepted as a potent approach to tackle almost all genetic diseases and as the next big thing in medicine. Although, not clinically applicable till now, it has clearly emerged as the last viable option for treatment. Genes carry all the genetic information and the information itself, can now be effectively used to treat its own disorders. After, Human Genome Project, there have been tremendous advances in DNA delivery and vector engineering technologies, to mitigate the defective gene and considerable success has been achieved in this regard. In future, elucidation of each gene's function and cross-liked metabolic pathways will unravel more intricacies involved and would help refine gene therapy strategies and in combination with traditional treatment approaches.

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